

We claim:

1. An isolated antibody, or an antigen-binding portion thereof, that dissociates from human erythropoietin receptor (EpoR) with a K_{off} rate constant of greater than about $1.3 \times 10^{-3} \text{ s}^{-1}$ and that activates an endogenous activity of said human EpoR in a mammal.
2. The antibody or antigen-binding portion thereof of claim 1 wherein said K_{off} rate constant is about $1.4 \times 10^{-3} \text{ s}^{-1}$ or greater.
3. The antibody or antigen-binding portion thereof of claim 1 wherein said K_{off} rate constant is about $1.9 \times 10^{-3} \text{ s}^{-1}$.
4. The antibody or antigen-binding portion of claim 1 wherein said K_{off} rate constant is about $4.8 \times 10^{-3} \text{ s}^{-1}$.
5. The antibody or antigen-binding portion of claim 1 wherein said K_{off} rate constant is determined by surface plasmon resonance.
6. The antibody or antigen-binding portion thereof of claim 1 wherein said antibody is a monoclonal antibody.
7. The antibody or antigen-binding portion thereof of claim 6 wherein said antibody is an IgG2 isotype.
8. The antibody or antigen-binding portion thereof of claim 1 that binds to human EpoR with a K_d of about 7 nM or greater.
9. The antibody or antigen-binding portion thereof of claim 8 wherein said K_d is about 8.5 nM or greater.
10. The antibody or antigen-binding portion thereof of claim 8 wherein said K_d is about 20 nM.

11. The antibody or antigen-binding portion thereof of claim 8 wherein said K_d is about 32 nM.

12. The antibody or antigen-binding portion thereof of claim 8 wherein said K_d is about 7-32 nM inclusive.

13. The antibody or antigen-binding portion thereof of claim 1 which is a human antibody.

14. An antibody or antigen-binding portion thereof that activates an endogenous activity of a human erythropoietin receptor in a mammal comprising a heavy chain variable region (HCVR) comprising an amino acid sequence of Formula I:

Y-I-X₁-X₂-X₃-G-S-T-N-Y-N-P-S-L-K-S (SEQ ID NO:18)

wherein:

X₁ is independently selected from the group consisting of tyrosine (Y), glycine (G) and alanine (A);

X₂ is independently selected from the group consisting of tyrosine (Y), glycine (G), alanine (A), glutamine (E) and aspartic acid (D); and

X₃ is independently selected from the group consisting of serine (S), glycine (G), glutamine (E) and threonine (T)

with the proviso that X₁-X₂-X₃ is other than Y-Y-S.

15. The antibody or antigen-binding portion thereof of claim 14 wherein X₁ is G and X₂ and X₃ are as defined therein.

16. The antibody or antigen-binding portion thereof of claim 14 wherein X₂ is G and X₁ and X₃ are as defined therein.

17. The antibody or antigen-binding portion thereof of claim 14 wherein X₃ is E and X₁ and X₂ are as defined therein.

18. The antibody or antigen-binding portion thereof of claim 14 wherein X_1 is G, X_2 is G and X_3 is as defined therein.

19. The antibody or antigen-binding portion thereof of claim 14 wherein X_1 is as defined therein, X_2 is G and X_3 is E.

20. The antibody or antigen-binding portion thereof of claim 14 wherein X_1 is G, X_2 is G and X_3 is E.

21. The antibody or antigen-binding portion thereof of claim 14 wherein X_1 is A, X_2 is G and X_3 is T.

22. An antibody or antigen-binding portion thereof comprising an amino acid sequence selected from the group consisting of

- (a) YIGGEGSTNYNPSLKS (SEQ ID NO:19);
- (b) YIAGTGSTNYNPSLKS (SEQ ID NO:20);
- (c) YIGYSGSTNYNPSLKS (SEQ ID NO:21);
- (d) YIYGSGSTNYNPSLKS (SEQ ID NO:22);
- (e) YIYYEGSTNYNPSLKS (SEQ ID NO:23);
- (f) YIGGSGSTNYNPSLKS (SEQ ID NO:24);
- (g) YIYGEGSTNYNPSLKS (SEQ ID NO:25); and
- (h) YIGYEGSTNYNPSLKS (SEQ ID NO:26).

23. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 1.

24. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering

to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 2.

25. A method of modulating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 3.

26. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 4.

27. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 6.

28. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 7.

29. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 8.

30. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 10.

31. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 11.

32. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 13.

33. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 14.

34. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 15, 16, 17, 18, 19, 20 or claim 21.

35. A method of activating an endogenous activity of a human erythropoietin receptor in a mammal, the method comprising the step of administering to said mammal a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 22.

36. A method of treating a mammal suffering aplasia, the method comprising the step of administering to a mammal in need of treatment a therapeutically effective amount of the antibody or antigen-binding portion thereof of claim 1.

37. A method of treating a mammal suffering aplasia, the method comprising the step of administering to a mammal in need of treatment a therapeutically effective amount of the antibody or antigen-binding portion thereof of claim 13.

38. A method of treating a mammal suffering anemia, the method comprising the step of administering to a mammal in need of treatment a therapeutically effective amount of the antibody or antigen-binding portion thereof of claim 1.

39. A method of treating a mammal suffering anemia, the method comprising the step of administering to a mammal in need of treatment a therapeutically effective amount of the antibody or antigen-binding portion thereof of claim 13.

40. A pharmaceutical composition comprising a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 1 and a pharmaceutically acceptable excipient.

41. A pharmaceutical composition comprising a therapeutically effective amount of an antibody or antigen-binding portion thereof of claim 13 and a pharmaceutically acceptable excipient.

42. An isolated or purified polynucleotide sequence which encodes a polypeptide comprising an amino acid sequence of Formula I:

Y-I-X₁-X₂-X₃-G-S-T-N-Y-N-P-S-L-K-S (SEQ ID NO:18)

wherein:

5 X₁ is independently selected from the group consisting of tyrosine (Y), glycine (G) and alanine (A);

X₂ is independently selected from the group consisting of tyrosine (Y), glycine (G), alanine (A), glutamine (E) and aspartic acid (D); and

10 X₃ is independently selected from the group consisting of serine (S), glycine (G), glutamine (E) and threonine (T)
with the proviso that X₁-X₂-X₃ is other than Y-Y-S.

43. The polynucleotide of claim 42 wherein X₁ is G and X₂ and X₃ are as defined therein.

44. The polynucleotide of claim 42 wherein X₂ is G and X₁ and X₃ are as defined therein.

45. The polynucleotide of claim 42 wherein X₃ is E and X₁ and X₂ are as defined therein.

46. The polynucleotide of claim 42 wherein X₁ is G, X₂ is G and X₃ is as defined therein.

47. The polynucleotide of claim 42 wherein X₁ is as defined therein, X₂ is G and X₃ is E.

48. The polynucleotide of claim 42 wherein X₁ is G, X₂ is G and X₃ is E.

49. The polynucleotide of claim 42 wherein X₁ is A, X₂ is G and X₃ is T.

50. The polynucleotide of claim 42 selected from the group consisting of

- (a) YIGGEGSTNYNPSLKS (SEQ ID NO:19);
- (b) YIAGTGSTNYNPSLKS (SEQ ID NO:20);
- (c) YIGYSGSTNYNPSLKS (SEQ ID NO:21);
- (d) YIYGSGSTNYNPSLKS (SEQ ID NO:22);
- (e) YIYYEGSTNYNPSLKS (SEQ ID NO:23);
- (f) YIGGSGSTNYNPSLKS (SEQ ID NO:24);
- (g) YIYGEGSTNYNPSLKS (SEQ ID NO:25); and
- (h) YIGYEGSTNYNPSLKS (SEQ ID NO:26).

51. A recombinant expression vector comprising the polynucleotide of claim 42.
52. A host cell comprising the recombinant expression vector of claim 51.
53. The host cell of claim 52 which is a eucaryotic cell.
54. The host cell of claim 52 which is a mammalian cell.
55. The host cell of claim 52 which is yeast cell.
56. The host cell of claim 52 which is a bacterial cell.
57. The host cell of claim 52 which is a CHO cell.
58. The host cell of claim 52 which is a COS cell.
59. The host cell of claim 52 which is an HEK-293 cell.
60. A polypeptide sequence encoded by said polynucleotide sequence of claim 42.
61. A polypeptide molecule comprising a first polypeptide sequence, a second polypeptide sequence, and a linking sequence, wherein:
said first polypeptide sequence is capable of binding a ligand;
said second polypeptide sequence is capable of binding a ligand;
said linking sequence connects said first polypeptide sequence and said second polypeptide sequence to form a single polypeptide chain; and
wherein said linking sequence comprises one or more amino acid sequences selected from the group consisting of, Gly-Phe-Lys-Asp-Ala-Leu-Lys-Gln-Pro-Met-Pro-Tyr-Ala-Thr-Ser (SEQ ID NO:37), Gly-His-Glu-Ala-Ala-Ala-

10 Val-Met-Gln-Val-Gln-Tyr-Pro-Ala-Ser (SEQ ID NO:4), Gly-Pro-Ala-Lys-Glu-Leu-Thr-Pro-Leu-Lys-Glu-Ala-Lys-Val-Ser (SEQ ID NO:3), and Gly-Glu-Asn-Lys-Val-Glu-Tyr-Ala-Pro-Ala-Leu-Met-Ala-Leu-Ser (SEQ ID NO:2).